

REMARKS

1. The Amendments and the Support Therefor

Claims 1, 14, 27 and 40 have been amended to leave claims 1-52 in the application. No new matter has been added by the amendments. For your convenience, following is a summary cross-referencing certain amendments to exemplary portions of the specification and/or drawings disclosing the recited structure:

<i>“DNA duplex”:</i>	support at e.g., page 3 line 27
<i>“double stranded DNA”:</i>	support at e.g., page 7 line 4
<i>“bound to a solid surface”:</i>	support at e.g., page 7 lines 4-5

2. Review of the Present Invention

Prior to reviewing the claim rejections, it is useful to review the present invention. The invention relates to the discovery that real-time tracking of probe hybridization to a target DNA molecule can be used for allelic discrimination (Dynamic Allele-Specific Hybridization or DASH). It was entirely unexpected that a system with the simplicity of DASH could provide sufficient resolution to unambiguously discriminate between all possible SNP variations using standardized conditions. The DASH system represents a considerable advance on previous allelic discrimination systems, which require lengthy optimization and expensive enzymatic or oligonucleotide labeling steps, and was the subject of a paper in the prestigious journal Nature Biotechnology (Howell et al (1999) Nature Biotechnology 17 87-88). (Note that this paper is later than the earliest claimed priority date and thus a copy is not submitted, though one could be submitted for the Examiner’s convenience at request.)

3. Sections 2-3 of the Office Action: Rejection of Claims 1, 3, 8, 10-12, 14, 16, 21, 23-25, 27, 29, 34, 36-38, 40, 42, 47 and 49-51 under 35 USC §102 in view of Ririe et al (Analytical Biochemistry (1997) 245 154-160)

Withdrawal of these rejections is requested. *Ririe et al* describes the use of an intercalating dye (SYBR Green I) to quantitate the production of specific PCR products. Different PCR

products in the same reaction can be distinguished by their different melting temperatures (itself governed by % GC content). There is no disclosure of a method of detecting DNA variation, as described in the present specification. The *Ririe et al* method lacks various features which are required by the present claims:

- In *Ririe et al*, PCR is conducted in solution. There is no disclosure of a single stranded DNA molecule attached to a solid surface, as recited in claim 1.
- There is no disclosure in *Ririe et al* of an oligonucleotide which is specific for one allele of a DNA variation. Contrary to the Examiner's assertion, there is no teaching in *Ririe* about using allele specific oligonucleotides to identify DNA variation through the absence of amplification. The teaching of *Ririe* is about distinguishing PCR products of different sequence using Tm. It is inherent in such a method that all products, whether or not they are mismatched, are amplified. Products cannot be distinguished on the basis of Tm if they have not been generated. The whole basis of the *Ririe* method is that amplification products are produced, and there is therefore a strong teaching away from using allele specific primers.
- Furthermore, an oligonucleotide must hybridize to the target sequence if an amplification product is to be generated. A method in which amplification failure is used to determine variations in the 3' terminus of the oligonucleotide primer, as proposed by the Examiner, would fall outside the present claims because there would be no hybridization of oligonucleotide to target DNA to form a complex.

The present claims specify a complex consisting of a single stranded DNA, oligonucleotide and marker and it is the formation or dissociation of this complex which is continually monitored. *Ririe et al* states at page 155 first column that fluorescence is measured as the reaction is heated to 95°C. What is being measured is the melting of a double stranded DNA molecule (i.e., the product of the previous round of amplification). This corresponds to a complex consisting of two single strands of a DNA sequence and a marker. There is no disclosure of monitoring the formation or dissociation of a complex consisting of a single stranded DNA, oligonucleotide and marker. The methods of *Ririe et al* therefore fall outside the present claims.

It is therefore submitted that *Ririe et al* does not teach the methods claimed in independent claims 1, 14, 27 and 40 (and thus their dependent claims 3, 8, 10-12, 16, 21, 23-25, 29, 34, 36-38, 42, 47 and 49-51), nor does it suggest the benefits of the claimed methodology, and withdrawal of the rejections is requested.

4. Section 4 of the Office Action: Rejection of Claims 1-5, 8, 10-18, 21, 23-31, 34, 36-44, 47 and 49-52 under 35 USC §102 in view of U.S. Patent 6,174,670 to Wittwer et al

The subject matter of *Wittwer et al* is an extension of the *Ririe et al* publication discussed in the foregoing Section 3 of this Response, and it relates to monitoring hybridization during PCR. *Wittwer et al* describes the use of double stranded DNA dyes, FRET pairs of oligonucleotide probes and single probes labeled with a FRET pair member along with target sequence labeled with the other FRET pair member.

The comments above in relation to *Ririe et al* also apply to those methods in *Wittwer et al* which involve double stranded DNA dyes such as SYBR Green I.

It is submitted that *Wittwer et al* does not anticipate the claims for at least the following reasons:

- The disclosure of *Wittwer et al* relates to methods of monitoring hybridization during PCR. PCR necessarily occurs in solution and there is no disclosure of a single stranded DNA molecule attached to a solid surface as recited in the present claims.
- The present claims recite a complex *consisting of* a single stranded DNA, oligonucleotide and marker, and it is the formation or dissociation of this complex which is continually monitored.
- Methods in *Wittwer et al* employing two FRET pair labeled probes which hybridize to a target strand in a FRET detection system (e.g. example 6) do not involve the formation and/or dissociation of such a complex because the complex formed in these methods consists of a target sequence, two oligonucleotide probes, an amplification primer, an extending complementary strand and a taq polymerase enzyme (see column 24 lines 21-24

and Example 3). Examples 5 and 6 explain the mechanism of action of the method and clearly indicate that probes are displaced by the polymerase (Examples 5 and 6), indicating that the complex containing the probes also contains the components described above.

- For similar reasons, methods in *Wittwer et al* employing a single probe labeled with a FRET pair member which hybridizes to a DNA strand comprising a primer labeled with the other member of the FRET pair do not fall within the present claims. The labeled probe hybridizes to the labeled target sequence to form a complex but this complex also contains an amplification primer, a nascent complementary strand and a taq polymerase enzyme. Thus, it is not a complex which falls within the definition provided by the present claims.

It is therefore submitted that *Wittwer et al* does not contain any teaching of the presently claimed methods, nor does it suggest that the claimed methods would be beneficially obtained, and thus the independent claims 1, 14, 27, and 40 (and their dependent claims) should be deemed allowable.

5. Sections 5-6 of the Office Action: Rejection of Claims 1-6, 8, 10-19, 21, 23-32, 34, 36-45, 47 and 49-52 under 35 USC §103(a) in view of Drobyshev et al (Gene 188 (1997) 45 52) and U.S. Patent 6,174,670 to Wittwer et al

As understood, the rejection alleges that a skilled person would be motivated to use the SYBR Green I marker of *Wittwer et al* in the methods of *Drobyshev et al* and this combination would fall within the present claims. Reconsideration and withdrawal of the rejections is requested because the methods of *Wittwer et al* and *Drobyshev et al* are very different in nature, and given these differences, one skilled in the art would not be motivated to combine these two disclosures as described by the Examiner. Example 2 of *Wittwer et al* describes the hybridization of 110 bp DNA duplex amplification products. *Drobyshev et al* describes the formation of non-amplified 10 bp DNA/RNA duplexes. SYBR Green I is said to be preferred for the methods of *Wittwer et al* because it provides great discrimination between double and single stranded DNA, is generally applicable to amplification reactions, and allows product specificity based on melting

curves. None of these alleged “advantages” are relevant to the methods of *Drobyshev et al.* The complexes of *Drobyshev et al* are not DNA duplexes and an increase in sensitivity is unlikely given the size of the complexes (10bp) relative to the *Wittwer et al* PCR products. Furthermore, monitoring occurs at a single temperature (T_d). Given the very different nature of the assays, it is submitted that there is no true and objectively ascertainable motivation for a skilled person to attempt to use SYBR Green 1 in a method according to *Drobyshev et al.*¹

Furthermore, even if a person of ordinary skill were motivated to combine *Wittwer et al* and *Drobyshev et al*, the skilled person would not arrive at the methods of the present invention. The oligonucleotides of *Drobyshev et al* are immobilized within a polyacrylamide gel. A polyacrylamide gel is neither solid nor is it a surface, as required by the present claims. In fact, it resembles more closely a liquid phase (*Drobyshev et al* page 48 section 2.3). The oligonucleotides are immobilized within the gel in a three dimensional manner, which is stated to allow a 100-fold greater capacity than a 2D surface such as glass. This enhances the sensitivity of the assay and the use of this gel is essential to the methods of *Drobyshev et al*. There is no disclosure of a single strand DNA sequence bound to a solid surface in *Drobyshev et al*, and based on the teaching of *Drobyshev et al*, there is no reason to expect that an oligonucleotide so bound would produce a detectable signal. This deficiency is not made up in *Wittwer et al*, which teaches that the strands of the PCR product are free in solution.

Furthermore, *Drobyshev et al* teaches that the oligonucleotide probe is immobilized and the sample (RNA fragments) is hybridized to it. The oligonucleotide probes are synthetic single stranded 10mer and are not derived from a double stranded molecule. The present claims require that it is the sample (single DNA strand of a double stranded DNA) which is immobilized. *Wittwer et al* provides no teaching on the subject as its teaching is directed at hybridization in solution. Therefore, neither *Wittwer* nor *Drobyshev* teach a “single DNA strand of a double stranded DNA containing the locus of a variation bound to a solid surface,” as recited by the

¹ To guard against hindsight, any suggestion to obtain the claimed invention must be “clear and particular” such that the motivation to combine or modify is evident (*Winner International Royalty Corp. v. Wang*, 53 USPQ2d 1580, 1586 (Fed. Cir. 2000)).

claims. An ordinary artisan could not arrive at this teaching from either document.

Furthermore, a method of *Drobyshev et al* which employed the SYBR Green I marker of *Wittwer et al* would involve the formation of an RNA/DNA heteroduplex complex between probe and target. *Drobyshev* discusses the advantages of such a complex on page 47 second column. By contrast, the present claims require a DNA homoduplex.

Furthermore, the methods of *Drobyshev et al* involve determining the output signal (fluorescence) at a single discriminatory temperature T_d to determine the state of dissociation/formation and thereby the presence of perfect or mismatched duplexes on the gel-chip(see table 2 and page 49 section 2.4). There is no recordation of the conditions of dissociation or formation as these conditions are predetermined as the T_d . By contrast, the present methods involve the continual measurement of the output signal and recording the conditions (e.g. the temperature) at which dissociation/formation occurs.

Other methods described in *Wittwer* describe the use of FRET. These methods appear to be incompatible with the methods of *Drobyshev et al* which involve the formation of small (10bp) heteroduplexes without amplification. It is not clear how these teachings could be combined, even if a skilled person could find motivation to try.

For the above reasons, a person of skill in the art would have no motivation to combine the disclosures of *Wittwer et al* and *Drobyshev et al* in the manner proposed by the Examiner. Furthermore, such a combination would not fall within the present claims. It is therefore submitted that the claimed methods are unobvious and thus should be allowed.

6. Section 7 of the Office Action: Rejection of Claims 1-8, 10-21, 23-34, 36-47 and 49-52 under 35 USC §103(a) in view of Drobyshev et al (Gene 188 (1997) 45 52), U.S. Patent 6,174,670 to Wittwer et al and U.S. Patent 6,048,690 to Heller et al

For the reasons previously described, *Drobyshev et al* and *Wittwer et al* do not teach the limitations of claims 1-6, 8, 10-19, 21, 23-32, 34, 36-45, 47 and 49-52. There is nothing in *Heller et al* which remedies the deficiencies of these disclosures. *Heller* describes the use of an electric field to induce perturbations in the fluorescence of a labeled probe hybridized to an

oligonucleotide which is bound to a chip via a biotin/streptavidin interaction. These fluorescent perturbations occur prior to "dehybridization" and are not attributable to the formation or disassociation of the hybridization complex. The use of intercalating dyes is proposed in column 9 line 57 to column 10 line 6. In these formats (see figures 3a and 4a), a biotinylated capture probe 8 is immobilized with streptavidin, a strand of target DNA 4 is hybridized to the capture probe 8 and a reporter probe 2 is then hybridized to the target DNA 4. The ethidium intercalates between the target DNA 4 and the reporter probe 2. It is noted that ethidium is not a suitable dye for use in the present methods (page 14 lines 24-27).

Furthermore, there is no disclosure of a complex *consisting of* a single DNA strand of a double stranded DNA containing the locus of a variation bound to a solid surface, an allele specific oligonucleotide probe, and a marker specific for the DNA duplex structure.

Thus, even if an ordinary artisan were motivated to make the attempt, the teachings of *Drobyshev et al*, *Wittwer et al* and *Heller et al* could not be combined by a person of ordinary skill in the art to arrive at the present invention. It is therefore submitted that the claimed methods are unobvious and these rejections should be withdrawn.

7. Section 8 of the Office Action: Rejection of Claims 1-6, 8-19, 21-32, 34-45 and 47-52 under 35 USC §103(a) in view of Drobyshev et al (Gene 188 (1997) 45 52), U.S. Patent 6,174,670 to Wittwer et al, and U.S. Patent 5,789167 to Konrad et al

For the reasons previously described, *Drobyshev et al* and *Wittwer et al* do not teach the limitations of claims 1-6, 8, 10-19, 21, 23-32, 34, 36-45, 47 and 49-52. *Konrad et al* does not remedy the deficiencies of these disclosures. *Konrad et al* describes the binding of a sample DNA to an immobilized capture oligonucleotide and the binding to the sample DNA of a probe oligonucleotide labeled with a fluorescent bead. Hybrid structures are analyzed in an electric field by fluorescence microscopy. The methods of *Konrad et al* do not involve the formation of a complex consisting of a) a single DNA strand of a double stranded DNA containing the locus of a variation bound to a solid surface, b) an allele specific oligonucleotide probe, and c) a marker specific for the DNA duplex structure, as described in the present specification and claimed.

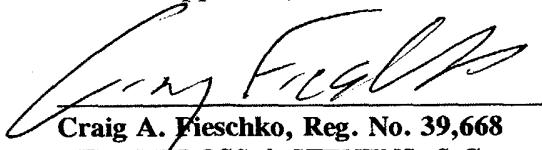
Konrad et al discusses Hepes buffers as a potential alternative to the exemplified Tris buffers. However, the present specification (page 17 lines 13-17) teaches that there are unexpected advantages to the use of Hepes buffers in the methods described. These advantages are not taught or suggested in *Konrad et al* and a person of ordinary skill in the art would have no motivation to use a Hepes buffer, as the skilled person would have no reason to anticipate any advantage in doing so.

Even if he were motivated to make the attempt, the teachings of *Drobyshev et al*, *Wittwer et al* and *Konrad et al* could not be combined by a person of ordinary skill in the art to arrive at the present invention. It is therefore submitted that the claimed methods are unobvious and these rejections should be withdrawn.

8. In Closing

If any questions regarding the application arise, please contact the undersigned attorney. Telephone calls related to this application are welcomed and encouraged. The Commissioner is authorized to charge any fees or credit any overpayments relating to this application to deposit account number 18-2055.

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ATTACHMENTS:

- Amendment Sheet ("Marked-Up" Copy) Showing Changes to Application

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No.: 09/755,747 Group Art Unit: 1637
Filing Date: January 5, 2001 Examiner: Freedman, J.
Applicant: Anthony J. Brookes Atty. Docket: 78104.017
Title: DETECTION OF NUCLEIC ACID POLYMORPHISM

**AMENDMENT SHEET ("MARKED-UP" COPY)
SHOWING CHANGES TO APPLICATION
(37 CFR §§1.121(b)(1)(iii); (c)(i)(ii))**

(To Accompany Response to 18 April 2002 Office Action)

In accordance with 37 CFR §§1.121(b)(iii) and (c)(ii), following are the amendments made to the specification and/or claims of the above-noted application.

- All deletions are indicated by brackets [like so] and all additions are indicated by underlining like so.
- The additions and deletions are made with respect to the application as it is understood to exist prior to entry of this amendment (i.e., any amendments are made with respect to the previous version).

IN THE CLAIMS:

Claims 1, 14, 27, and 40 are amended as follows:

1. **[AMENDED]** A method of detecting DNA variation by monitoring the formation or dissociation of a complex consisting of:
 - (a) a single DNA strand of a [DNA sequence] double stranded DNA containing the locus of a variation bound to a solid surface,
 - (b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a DNA duplex,
 - (c) a marker specific for the DNA duplex structure of (a) plus (b) which forms a complex with the said duplex and reacts uniquely when interacting within the DNA duplex,which method comprises:
 - (1) continually measuring an output signal indicative of interaction of the marker with duplex formed from the strand (a) and probe (b), and
 - (2) recording the conditions at which a change in reaction output signal occurs which is attributable to formation or dissociation of the complex and is thereby correlated with the strength with which the probe (b) has hybridized to the single strand (a).

14. [AMENDED] A method of detecting DNA variation which comprises bringing together:

- (a) a single DNA strand of a [DNA sequence] double stranded DNA containing the locus of a variation bound to a solid surface,
- (b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a DNA duplex,
- (c) a marker specific for the DNA duplex structure of (a) plus (b) which forms a complex with the said duplex and reacts uniquely when interacting within the duplex,

[wherein:

- (1) **bringing together]**
thereby forming a complex consisting of the components (a), (b) and (c), wherein the components (a), (b), and (c) are brought together under conditions in which either
 - (i) the component (a) hybridizes to component (b) and the complex is formed with component (c), or
 - (ii) the components (a) and (b) do not hybridize and the complex with component (c) is not formed,
- (2) thereafter steadily and progressively adjusting the conditions of the environment, respectively, either
 - (i) to denature the formed DNA duplex and cause dissociation of the complex, or
 - (ii) to cause formation of the DNA duplex and resulting complex,
- (3) continually measuring an output signal indicative of the extent of hybridization of (a) and (b) and resulting complex formation with (c), and
- (4) recording the conditions in which a change of output signal occurs which is indicative of, respectively,
 - (i) dissociation of the complex, or
 - (ii) formation of the complex.

27. [AMENDED] A method of detecting DNA variation which comprises:

- (1) forming a complex consisting of:
 - (a) a single DNA strand of a [DNA sequence] double stranded DNA containing the locus of a variation bound to a solid surface,
 - (b) an oligonucleotide or DNA analogue probe specific for one allele of the variation hybridized to the single strand (a) to form a duplex, and
 - (c) a marker specific for the DNA duplex structure of (a) plus (b) and which reacts uniquely when interacting within the DNA duplex, and
- (2) continually measuring an output signal of the extent of the resulting reaction of the marker and the duplex while steadily increasing the denaturing environment containing the complex,
- (3) recording the conditions at which a change in reaction output signal occurs (herein termed the denaturing point) which is attributable to dissociation of the complex and is thereby correlated with the strength with which the probe (b) has hybridized to the single strand (a).

40. [AMENDED] A method of detecting DNA variation which comprises:

- (1) bringing together:
 - (a) a single DNA strand of a [DNA sequence] double stranded DNA containing the locus of a variation bound to a solid surface,
 - (b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a DNA duplex,
 - (c) a marker specific for the DNA duplex structure of (a) plus (b) and which reacts uniquely when interacting within the duplex,

the components (a), (b) and (c) being brought together prior to formation of the defined complex and under conditions in which (a) and (b) do not hybridize;
- (2) steadily adjusting the conditions of their environment to cause formation of the duplex and resulting complex consisting of components (a), (b), and (c), and
- (3) measuring an output signal indicative of the occurrence of hybridization of (a) and (b) (herein termed the annealing point).